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Research Article
Characterization of Anti-oxidant and Anti-microbial Properties of *Tamarindus indica* Bark Extract on Various Pathogenic Micro-organisms

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Abstract

Background and Objectives: Prevention of food spoilage is usually achieved by use of chemical preservatives which have negative impacts human health. Due to such concerns it is necessary to find some natural preservatives. Within this text, the present study was designed to investigate the *in vitro* anti-microbial activities, MIC, MBC, phytochemical analysis and anti-oxidant activity of the acetone extract of bark of *Tamarindus indica* against the pathogenic micro-organisms. **Materials and Methods:** The *in vitro* anti-bacterial and anti-fungal activity was performed by agar well diffusion method while the phytochemical analysis was investigated by different methods like Mayer's test, Ninhydrin test, Alkaline Reagent test, Ferric Chloride test, Froth test, Xanthoprotic test and Liebermann Burchard test. Antioxidant property was evaluated by DPPH method. **Results:** The *T. indica* bark shows the presence of phytochemicals like, phenolic compounds, tannins and steroids. The anti-microbial activity results were expressed as the average diameter of zone of inhibition. The acetone extract showed better anti-bacterial activity and with zone of inhibition (28-30 mm) and showed no anti-fungal activity when compared with other tested standard anti-biotic streptomycin (300 ppm) and trizol solution. The MIC of the acetone extracts ranged from 3.125-6.25 μg mL^{-1}. However, MBC ranges from 6.25-12.5 μg mL^{-1} Extracts of *T. indica* bark also exhibited significant anti-oxidant activity, thus establishing the extracts as an anti-oxidant. **Conclusion:** The results obtained in this study give some scientific support to the *T. indica* bark for further investigation of compounds and in future could be used as drug and combinations of extracts may be the best herbal antibiotics.

Key words: *Tamarindus indica*, phytochemicals, anti-bacterial, anti-fungal, MIC, MBC, anti-oxidant

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food products can be contaminated by a variety of pathogenic and spoilage microbiota, the former causing food borne diseases and the latter causing significant economic losses for the food industry due to undesirable effects on the food properties. Chemical additives have been extensively used to prevent the survival and proliferation of microorganisms but their safety and impact on human health are under discussion. In packaged foods, growth and survival of common spoilage and pathogenic micro-organisms such as *Listeria monocytogenes*, *Escherichia coli* O157, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter*, *Clostridium perfringens*, *Aspergillus niger* and *Saccharomyces cerevisiae* are affected by a variety of intrinsic factors, such as pH and presence of oxygen or by extrinsic factors associated with storage conditions, including temperature, time and relative humidity[1-3]. The prevention or inhibition of microbial growth in foods is of outmost importance for the current globalized food production. Hence, there is still the need for new processing methods or some natural anti-microbials which are able to reduce or eliminate foodborne pathogens and spoilage bacteria to prevent the economic losses of food industry and human health. Traditionally crude plant extracts are used as herbal medicine for the treatment of infectious diseases because of the presence of phytochemical. The phytochemicals work in the human system and due to their therapeutic properties cure many ailments which cannot be cured by the modern drugs[4]. The anti-microbial properties of natural substances such as plant essential oils and extracts have been extensively studied with promising results[5]. A natural source for developing antimicrobials includes plants and their parts and these could be used to control the diseases caused by pathogenic microbes[6].

Tamarindus indica, (Tamarind), family, Leguminosae, is one such widely used medicinal plant. It is found in virtually all tropical climatic regions, from India through Africa to the Caribbean and South America and up to southern Florida. Its uses are as varied as the cultures that use it. It is often more difficult to determine which use is more important, as food and beverage[7,8]. In northern Nigeria, the fresh stem bark and fresh leaves are used as decoction mixed with potash for the treatment of stomach disorder, general body pain, jaundice, yellow fever and as blood tonic and skin cleanser[9]. Development of antibiotic resistance in bacteria is a major issue in the prevention of infectious diseases[10,11]. Therefore, antibacterial agents are needed to be developed and employed to control multi-drug resistant bacteria. There has been growing interests to find antibacterial compounds from plant extracts as an alternative approach to deal with this problem[11-14]. It is necessary to carry out detail examination of novel antimicrobials which have no large toxic effects as well as shows effectiveness against drug resistant bacteria. In this category, bacteria include *Escherichia coli*, *Pseudomonas aeruginos*, *Staphylococcus aureus*, *Salmonella*[15,16].

The present study aimed at evaluating the anti-microbial activities, MIC, MBC, phytochemical analysis and anti-oxidant activity of the acetone extract of bark of *Tamarindus indica* against the pathogenic micro-organisms such as *Salmonella* sp., *Staphylococcus aureus*, *Pseudomonas aerogenosa*, *Escherichia coli*, *Candida albicans* and *Aspergilus niger*.

MATERIALS AND METHODS

Collection of plant material: Bark of *Tamarindus indica* was collected from herbal garden of IFTM University, Moradabad, district of Uttar Pradesh, India. This plant was identified and confirmed with the authentic sources. Fresh bark were washed thoroughly under running tap water and dried under shade. They were then finely ground to a powder in an electric blender. All parts were extracted with acetone using soxhlet apparatus[17]. After removal of solvents under reduced pressure, extracts were stored at -20°C for further use. Then the extracts were used for phytochemical analysis, anti-bacterial and anti-fungal activity.

Phytochemical analysis

Mayer's test: Mayer's test was performed for the presence of alkaloids. About 1 mL plant extract was taken and added 3 mL of ammonia solution. The solution was allowed to stand for few minutes to evaluated free alkaloids. About 10 mL of chloroform was added to the test tube, shaken by hand and then filtered. The chloroform was evaporated from the crude extract by water bath. About 3 mL of Mayer's reagent was added. A cream colour precipitation was obtained immediately that showed the presence of alkaloids[18].

Ninhydrin test: For amino acid detection acetone extract was treated with 0.25% w/v ninhydrin reagent and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Alkaline reagent test: For the presence of flavonoids in plant extract, 1 mL plant extract was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid that indicated the presence of flavonoids[19].

Keller-Killiani test: For the qualitative analysis of glycosides, test solution was treated with few drops of glacial acetic acid and ferric chloride solution and mixed. Concentrated sulphuric acid was added and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides[20].

Ferric chloride test: For the presence of phenolic compounds extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols[19].

Xanthoprotic test: Presence of protein content was confirmed by this method. The extracts were treated with few drops of conc. HNO_3. Formation of yellow color indicates the presence of proteins.

Froth test: Presence of saponins was confirmed by Froth test. Extracts were diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

Liebermann burchard test: About 1 mL extracts was taken in a test tube and dissolved with chloroform (10 mL). Then add equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids[21].

Gelatin test for tannins: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Anti-bacterial activity: Agar well diffusion method elucidated by Azam *et al.*[22] was employed in present study.

The pure culture of organism was sub-cultured in nutrient broth the nutrient agar plates, prepared by 25 mL (for one plate) of molten media into sterile petriplates. For bacterial growth, a lawn of culture was prepared by spreading the 100 µL fresh culture having of each test organism on nutrient agar plates with the help of a sterile glass rod spreader. Plates were left standing for 10 min to let the culture get absorbed. Then 6 mm (size) wells (2 well) was punched into nutrient agar plates for testing extract antibacterial activity. Using the micro-pipette, 20 µL of sample suspension was poured in into one well on the plates. The antibiotic, streptomycin of 300 mg was prepared in 1000 mL acetone. This antibiotic was used as positive control. Using the micropipette, 20 µL of antibiotic (control) solution was poured into one well. After incubation for 24 h at 37°C the plates were observed. Anti-bacterial activity was shown on the plates by presence of an inhibition zone surrounding the well. The zone of inhibition was measured and expressed in millimeters.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): To determine the Minimum Inhibitory Concentration (MIC), dilution of the extract of was prepared in sterile nutrient broth to achieve a decreasing concentration ranging from 100-50 µL mL^{-1} in sterile tubes labelled 1-6. For determination of Minimum Inhibitory Concentration (MIC) defined as the lowest concentration of material that inhibits the growth of an organism was a determined based on batch culture containing varying concentration of extract in suspension (20-300 mg L^{-1}). Sterile 6 test tubes were taken, each containing 1 mL nutrient broth after adding the extract to prevent aggregation of the nanoparticles. All sample tube and were serially diluted subsequently, the test tube was inoculated with 1 mL of the freshly prepared bacterial suspension and then incubated at 37°C for 24 h. The experiments also included a positive control (flask containing nanoparticles and nutrient media, devoid of inoculum) and a negative control (flask containing inoculum and nutrient media, devoid of nanoparticles). The negative controls indicated the microbial growth profile in the absence of nanoparticles. The absorbance values for positive controls were subtracted from the experimental values (flasks containing nutrient media, inoculum and nanoparticles). Extract would be tested for bactericidal effect using all the microbial cultures selected for the study. The concentration of tube without visible growth of the bacterial cells was the MIC.

To evaluate the MBC, 100 µL of sample from each tube without visible growth was transferred into nutrient agar plate and then incubated aerobically for another 24 h. The concentration of the tube without growth was the MBC (in this test, the population in agar plate less than 10 was regarded no growth). The Minimum Bactericidal Concentration (MBC), i.e., the lowest concentration of extract that kills 99.9% of the bacteria also determined from the batch culture studies. The extract concentration causing bactericidal effect will be selected based on absence of colonies on the agar plate.

RESULTS

The acetone extract of bark of *T. indica* showed various levels of anti-bacterial activity when tested by well diffusion method. The antibacterial susceptibility of the acetone extract of bark of *T. indica* on various micro-organisms

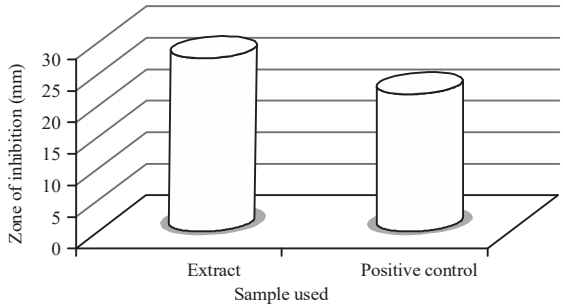

Fig. 1: Graphical representation of anti-bacterial activity of acetone extract of *T. indica* with positive control (Streptomycin) against *P. aeruginosa*

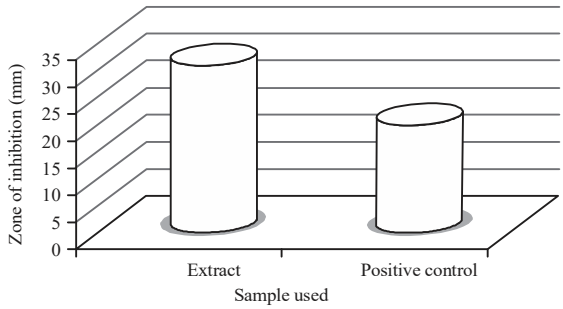

Fig. 2: Graphical representation of anti-bacterial activity of acetone extract of *T. indica* with positive control (Streptomycin) against *S. aureus*

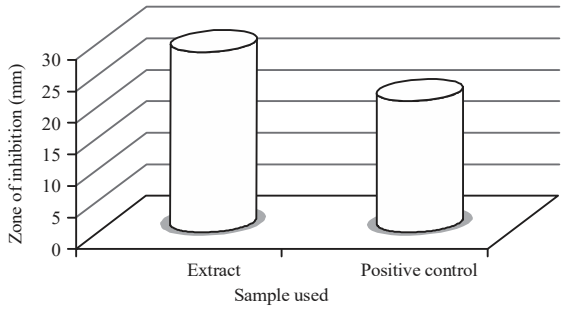

Fig. 3: Graphical representation of anti-bacterial activity of acetone extract of *T. indica* with positive control (Streptomycin) against *E. coli*

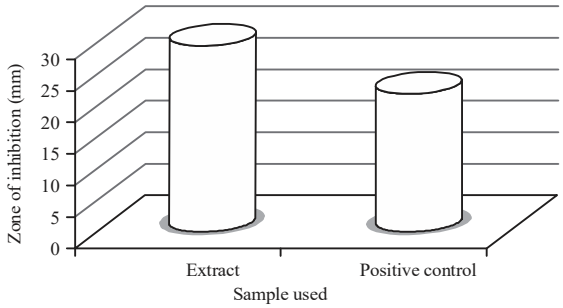

Fig. 4: Graphical representation of anti-bacterial activity of acetone extract of *T. indica* with positive control (Streptomycin) against *Salmonella* spp.

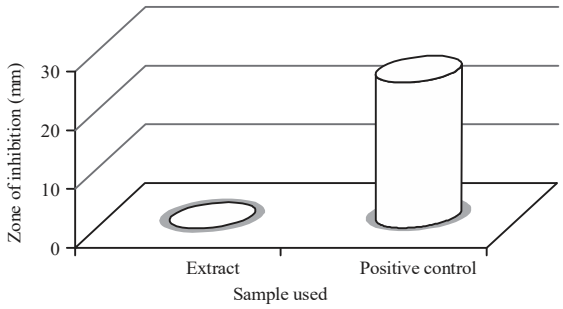

Fig. 5: Graphical representation of anti-fungal activity of acetone extract of *T. indica* with positive control (Trizol Solution) against *C. albicans*

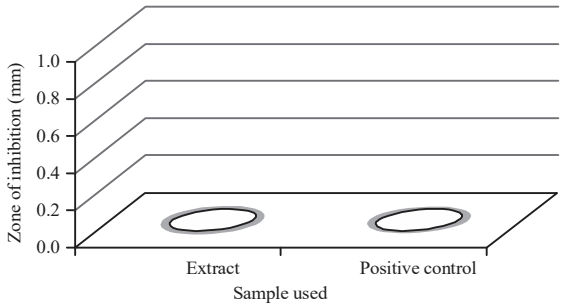

Fig. 6: Graphical representation of anti-fungal activity of acetone extract of *T. indica* with positive control (Trizol Solution) against *A. niger*

like *Pseudomonas aerugenosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp. The anti-bacterial activity of *T. indica* against on, *Staphylococcus aureus* and *Salmonella* showed that highest zone of inhibition i.e., 30 mm. The anti-bacterial activity against on *E. coli* showed the zone of inhibition i.e., 29 mm. The anti-bacterial activity against on *Pseudomonas aerugenosa* showed the zone of inhibition i.e., 28 mm. The anti-fungal activity of acetone extract of bark of *T. indica* against on *Candida albicans* and *Aspergillus niger* showed no zone of inhibition (Fig. 1-6).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): For the estimation of MIC and MBC 6 different concentrations (25, 12.5, 6.25, 3.125, 1.562 and 0.781 μg mL^{-1}) were used against bacterial strain B1 (*P. aeruginosa*); B2 (*S. aureus*); B3 (*E. coli*) and incubated at 37°C, 48 h.

Anti-oxidant activity: Antioxidant activity, performed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method[23] of acetone extract of *T. indica* bark were found

Table 1: Phytochemical analysis of acetone extract of *T. indica* bark

Test name	Method used	Result
Alkaloids test	Mayer's test	-ve
Amino acids test	Ninhydrin test	-ve
Glycosides test	Keller-Killiani test	-ve
Phenols test	Ferric Chloride test	+ve
Proteins test	Xanthoprotic test	-ve
Saponins test	Froth test	-ve
Steroids test	Liebermann Burchard test	+ve
Tannins test	Gelatin test	+ve

Table 2: Anti-bacterial activity of acetone extract of *T. indica* bark

Test micro-organism	T.I. (mm)	+ve control (mm)
P. aeruginosa	28	22
S. aureus	30	20
E. coli	29	21
Salmonella	30	22

Positive control used: Streptomycin (300 ppm), Well size: 6 mm (excluded from zone of inhibition)

Table 3: Anti-fungal activity of *T. indica* bark acetone extract

Test microorganism	*T. indica* (mm)	+ve control (mm)
Candida albicans	NIL	25
Aspergillus Niger	NIL	NIL

Positive control used: Trizol solution, Well size: 6 mm (excluded from zone of inhibition)

Table 4: MIC (mg mL^{-1}) and MBC (mg mL^{-1}) values for the acetone extract of *T. indica* bark against test organism

	MIC (µg mL^{-1})				MBC (µg mL^{-1})			
Extract	B1	B2	B3	B4	B1	B2	B3	B4
T. indica	6.25	3.125	3.125	3.125	12.25	6.25	6.25	6.25

B1: *P. aeruginosa*, B2: *S. aureus*, B3: *E. coli*, B4: *Salmonella* sp.

12.7% increase in DPPH radical scavenging activity at 5 µg mL^{-1} concentration shows moderate level of scavenging activity of extract.

DISCUSSION

The phytoconstituents analysis of tamarind in the present study reviles the similarities with previously reported study by Doughari[9], Daniyan and Muhammad[24] and Subramanya and Muttagi[25]. In present study an attempt was made to characterize the phytochemical, anti-oxidant and anti-microbial properties of *Tamarindus indica* acetonic extract of bark and their synergistic effect to prolong the shelf life of fruits and food stuff. The results obtained from the phytochemical, anti-microbial activity of the bark extracts of *T. indica* shown in Table 1-3 shows that it could be a potent anti-microbial activity on the tested micro-organisms. However, the bark extracts were also found to have the highest activity against *Salmonella* and *Staphylococcus* with the range of 30 mm zone of inhibition. But interestingly it was found that acetonic extract of bark showed no inhibitory effect on pathogenic strain of fungus *Aspergilus niger* and *Candida albicans*. However, tamarinds pulp is used as preservatives for food stuff to prolong the shelf life were reported earlier[26]. From the overall anti-microbial activity of the plant, it has been seen that the effects of the extracts are concentration dependent and this shows that at higher concentrations, more effect (antimicrobial activity) is expected. From the assay of the MIC and MBC of the extracts of *T. indca* (Table 4) the results shows that the plants extracts can be both bacteriostatic and bactericidal[27-29]. Anti-oxidant activity is one of the key properties for designer food on the basis of their role in human health specially due to presence of free radical scavenging activity and protection against oxidative stress[30,31] and thus provides resistance towards bacterial infections among the processed food materials. Anti-oxidant properties play a pivotal role for the cure of heart disease and cancer[32,33]. The acetone extract of *T. indica* bark were found 12.7% increase in DPPH radical scavenging activity at 5 µg mL^{-1} concentration shows moderate level of scavenging activity of extract.

CONCLUSION

The anti-bacterial activity of *T. indica* demonstrated by tamarind that the bark extracts could be used to control bacterial growth among food materials and to develop a quality food products with prolong shelf life along with antioxidant properties. The synergistic effect of anti-bacterial with anti-oxidant properties can be the basis to include bark extract of *T. indica* as food additive to prolong self-life of food with scavenging properties of oxidative stress. The finding from this present study shows a remarkable property of tamarind bark extract and it could be exploit for possible phytogenic agent to control *Staphylococcus aureus* and *salmonella* spp. infections in food materials. However, further study has to be needed for quantitative analysis on the concentration of tamarind extracts that could be the effective food additives as synthetic antibiotics to develop quality food products. Further work may be extended by the authors to identify the bioactive compound in bark extract of *T. indica* using advanced instrumentation techniques.

SIGNIFICANCE STATEMENT

The present study has demonstrated that the acetone extract of *T. indica* bark could be a better alternate for antibacterial activity along with antioxidant scavenging

properties act as an option to include as food additives for the category of designer food formulations and for drug formulation.

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